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is plasticity between these states, has important implications for efforts to preserve islet functionality in diabetes.

Hubs are important; but are they also a weak link in islet function? Beta cell diversity was previously shown to be clinically relevant since insults may preferentially target specific cell populations to induce beta cell failure (Rutter and Hodson, 2015). In the current work, Johnston et al. (2016) show that acutely challenging mouse or human islets with pro-inflammatory cytokines or a glucolipotoxic milieu reduces the number of hubs, which is consistent with the hypothesis that hub cells may be especially vulnerable. However, Johnston et al. (2016) did not examine whether loss of connectivity consequently reduced insulin secretion, or alternatively whether dysfunctional diabetic human islets also show loss of hubs. Furthermore, it is unclear whether non-hub beta cells are also sensitive to these insults, or whether hubs represent the islet's Achilles' heel. One would expect hub cells with <20% of the normal levels of Pdx1 to be highly susceptible to apoptosis (Johnson et al., 2003). In vivo studies will require new markers to understand how hubs and their followers change over longer periods of time and how the hub-follower ratio affects glucose homeostasis. Unbiased discovery of hub markers in the future may be possible with light-dependent CRISPR-mediated genomic barcoding and single-cell transcriptomics.

In the meantime, Johnston et al. (2016) have made a major breakthrough in islet biology and established an exciting new research field. It is remarkable that such a small number of beta cells exerts such dominant control over connectivity and islet function.

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Fueling Performance: Ketones Enter the Mix

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Ketone body metabolites serve as alternative energy substrates during prolonged fasting, calorie restriction, or reduced carbohydrate (CHO) availability. Using a ketone ester supplement, Cox et al. (2016) demonstrate that acute nutritional ketosis alters substrate utilization patterns during exercise, reduces lactate production, and improves time-trial performance in elite cyclists.

Exercise intensity is the primary determinant of substrate utilization during exercise. As intensity increases, the contribution of substrates to energy provision shifts from blood-borne free fatty acids (FFAs) and glucose toward increased reliance on intramuscular triglyceride (IMTG) and glycogen (Egan and Zierath, 2013). At moderate-to-high exercise intensities (>75% of maximal oxygen uptake, VO_2max), muscle glycogen is the main source of energy provision. This hierarchy of fuel selection in exercising muscle is long established, and, consequently, nutrition strategies for fueling for most competitive athletic performance are based around optimizing CHO provision before and during performance. Nevertheless, training with reduced CHO intake may be a means to improve body composition and enhance metabolic and mitochondrial adaptations to exercise (Burke, 2015). Moreover, there is increasing interest in the purported benefits of ketogenic diets (KDs) and the use of exogenous ketone supplements within the athletic community, particularly in ultra-endurance sports. Using a ketone ester food supplement initially developed for enhancing warfighter performance, the work by Clarke and colleagues (Cox et al., 2016) comprises a series of experiments employing acute ingestion of ketone ester by highly trained cyclists prior to and during intense cycling exercise, while examining outcomes related to fuel selection, mitochondrial bioenergetics, intramuscular substrate utilization, and time-trial performance. The provocative findings

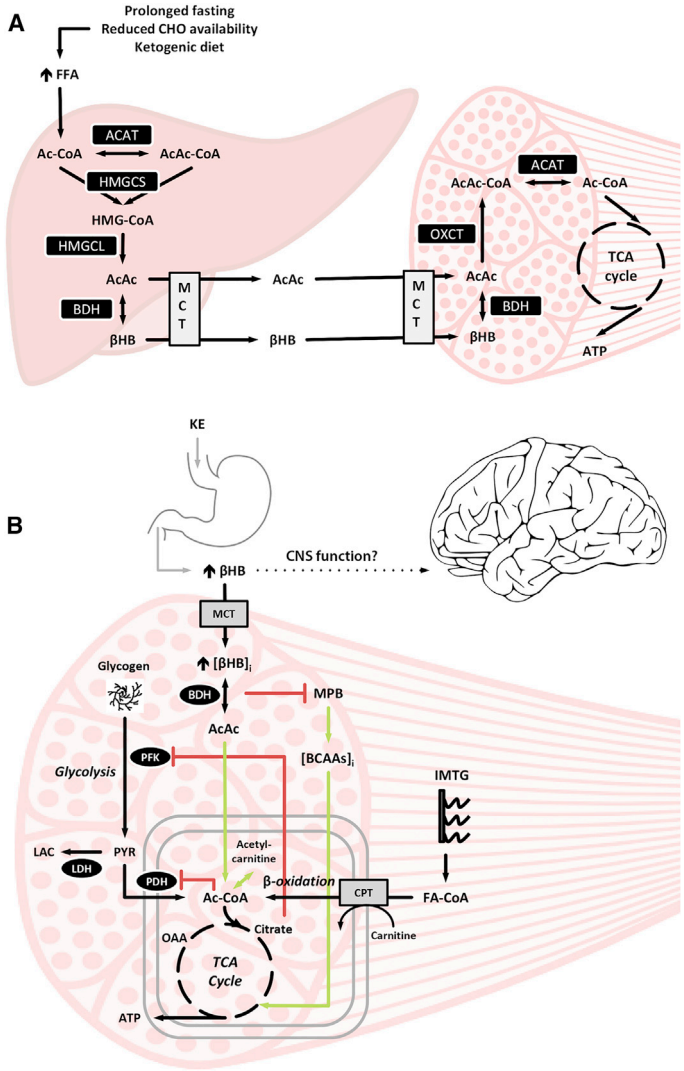

Figure 1. Pathways of Ketogenesis in Liver and Ketolysis in Skeletal Muscle and Proposed Mechanisms of Regulation of Fuel Selection and Performance by Exogenous Ketones

(A) Ketogenesis is an evolutionarily conserved adaptive response crucial for survival during an energy crisis by ensuring substrate for brain, which cannot utilize FFAs as a fuel source. Ketogenesis involves sequential reactions of condensation of acetyl-CoA (Ac-CoA) molecules to acetoacetyl-CoA (AcAc-CoA) by mitochondrial thiolase activity of Ac-CoA acetyltransferase (ACAT), generation of hydroxymethylglutaryl-CoA (HMG-CoA) by hydroxymethylglutaryl CoA synthase (HMGCS), and decomposition of HMG-CoA, liberating AcAc and Ac-CoA, in a reaction catalyzed by HMG-CoA lyase (HMGCL). Some AcAc will be exported to the circulation, but the majority is reduced to βHB by β-hydroxybutyrate dehydrogenase (BDH). Transport of ketones bodies into and out of the circulation occurs via monocarboxylate transporters (MCTs) in mitochondrial and sarcolemmal membranes. The only metabolic fate of βHB is inter-conversion with AcAc, and upon entry into peripheral tissues it is re-oxidized to AcAc by BDH. For ketolysis, covalent activation of AcAc by CoA is catalyzed by succinyl-CoA:3-oxoacid CoA transferase (OXCT), resulting in generation of AcAc-CoA. Two molecules of Ac-CoA are liberated by thiolytic cleavage of AcAc-CoA by ACAT, after which Ac-CoA is incorporated into the TCA cycle. These are incorporated into the TCA cycle via citrate synthase for terminal oxidation and production of ATP, which in skeletal muscle contributes to fueling muscular work.

(B) KBs can be utilized as a fuel source by peripheral tissues including muscle, but also exert a range of metabolic effects including anti-catabolic effects on muscle and attenuation of glucose utilization and lipolysis. The authors demonstrate rapid bioavailability of KE (573 mg/kg) with βHB rising to ~6 mM at 30 min after ingestion at rest. Acute nutritional ketosis (~3 mM) during exercise attenuated muscle protein breakdown (MPB), muscle glycogen utilization, glycolytic flux and lactate (LAC) appearance, and increased IMTG utilization. These effects on glycolytic flux are likely mediated inhibition of PDH and phosphofructokinase (PFK) by elevations in Ac-CoA and citrate formation, respectively, as a consequence of metabolism of AcAc. Moreover, given the well-established influence of KBs on CNS function, future work examining potential effects during exercise is warranted. CPT, carnitine palmitoyltransferase; LDH, lactate dehydrogenase; OAA, oxaloacetate; PYR, pyruvate.

challenge existing paradigms in exercise metabolism and leave several exciting questions to be answered by future work.

The ketone bodies (KBs), namely β-hydroxybutyrate (βHB) and acetoacetate (AcAc), are produced by ketogenesis in the liver in order to preserve brain metabolism and fuel heart and skeletal muscle when CHO availability is restricted (Figure 1A). KBs are utilized by skeletal muscle during exercise (Balasse and Féry, 1989), and their uptake and oxidation during exercise are enhanced by exercise training (Winder et al., 1975). These, in addition to a glucose-sparing action in skeletal muscle (Robinson and Williamson, 1980) and potential to lower the exercise-induced rise in plasma lactate (Balasse and Féry, 1989), suggest potential performance benefits of KBs if provided as an exogenous fuel source, but this has received little attention to date. The development of ketone esters provides a novel method to increase βHB and achieve acute nutritional ketosis while circumventing impractical dietary restrictions. Acute ingestion of either the *R,S*-1,3-butanediol acetoacetate diester (D'Agostino et al., 2013) or the *(R)*-3-hydroxybutyl *(R)*-3-hydroxybutyrate ketone monoester (KE) (Cox et al., 2016) can result in short-term (~15 min to 6 hr) nutritional ketosis indicated by elevated (>1 mM) βHB and AcAc.

In their first experiment, the authors demonstrate that during exercise lasting 45 min at either 40% or 75% of maximal power output (W_{max}), βHB was ~2 and 3 mM, respectively, lower than ketosis produced after ingestion at rest, i.e., intensity-dependent utilization of βHB during exercise was observed. Based on expired air analysis adjusted for oxidation of KBs, βHB oxidation contributed 16% to 18% of oxygen consumption to energy provision during exercise. Previously reported contributions of KB oxidation to energy provision have typically been 2% to 10%, but the greater contribution herein likely reflects a greater capacity of skeletal muscle to uptake and utilize KBs in elite athletes. Additionally, acute nutritional ketosis is markedly different from the fasting-induced ketonemia employed in previous exercise studies (Balasse and Féry, 1989). In the latter, elevated KB can act in a self-regulating manner to impair KB oxidation in skeletal muscle in

order to maintain KB availability to the brain.

Cox and colleagues also demonstrate attenuation (~50%; ~2 to 3 mM) of the rise in plasma lactate during exercise at 75% W_{max} after KE ingestion compared to ingestion of an isocaloric CHO drink. Subsequent experiments demonstrated inhibition of glycolytic metabolism and sparing of muscle glycogen, reduction in protein degradation, and elevated IMTG utilization during exercise after KE ingestion. Thus, acute nutritional ketosis via exogenous ketones modulates skeletal muscle metabolism during exercise, particularly by reducing the contribution of anaerobic pathways to energy provision at intensities above lactate threshold (Figure 1B). Lastly, after a 60 min pre-load at 75% W_{max}, cycling performance in a 30 min time trial improved by 2% (411 ± 162 m; mean ± SEM, n = 8) compared to CHO ingestion. This was a result of a fueling strategy that combined KE (40%) with CHO (60%) and elevated βHB to between ~1.5 and 3.0 mM throughout.

These eye-catching data suggest that exogenous ketones can confer a performance benefit to elite athletes through a combination of fuel sparing and improved energetic efficiency. However, despite the notable effects on fuel selection and CHO sparing during steady-state exercise, muscle glycogen depletion would not be expected to be limiting during such a performance test. Alternatively, reduced glycolytic flux and lactate production after KE ingestion may attenuate accumulation of hydrogen ion and metabolic by-products that are among a myriad of factors that contribute to fatigue during intense exercise. If reduced glycolytic flux is a result of impairment to glycolysis, conversely, this may be deleterious for sports that are intermittent and/or require periods of high-intensity "bursts" that rely heavily on contributions from glycolytic pathways. For example, when athletes undertake KDs and variants, impaired performance during intermittent high-intensity efforts can occur and may be explained by sustained attenuation of pyruvate dehydrogenase (PDH) activity (Burke, 2015). On the other hand, the often-cited higher energetic efficiency of exogenous ketones may provide thermodynamic advantages over CHO and fat given the greater free energy of ATP hydrolysis ($\Delta G'_{ATP}$) and less oxygen required per mole of carbon. In practical terms, this would translate as a higher power output for the same oxygen consumption (i.e., improved muscular efficiency) during exercise, and thereby confer a performance benefit as observed.

While a 2% improvement in performance may appear modest, in elite sport, the difference between winning and losing is often less than that (Malcata and Hopkins, 2014). However, in laboratory tests of performance such as those employed by Clarke and colleagues, the coefficient of variation is typically 1% to 3%. Tests of ergogenic potential must account for this variation, such that when improvements observed are near the limits of sensitivity for a performance test, some caution should be exerted. Additionally, although efforts are made to blind participants to experimental conditions, placebo effects in sports performance are often similarly 1% to 3% (Beedie and Foad, 2009). Our experience is that it is extremely difficult to mask or even mimic the distinct taste of KEs, which could be an issue for those familiar with the taste by experience or reputation. Given the well-established effects of KBs on CNS function, potential performance benefits related to motor recruitment, perceived exertion, pacing strategies, skill execution, reaction time, and decision making will be interesting for future research (Figure 1B). As there is also a proposed role for the CNS in regulating performance beyond effects related to skeletal muscle metabolism (Noakes, 2011), clearly a multi-organ perspective on ergogenic potential of exogenous ketones is warranted. Additionally, pharmacokinetic evaluation of current and emerging exogenous ketone supplements in humans will inform optimal dosing strategies and protocols for physical and cognitive resilience, exercise performance, and recovery.

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